

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Martha G. Welch et al. Confirmation No.: 8041
Application No.: 10/799,941 Art Unit: 1654
Filed: March 11, 2004 Examiner: Andrew D. Kosar
Title: NOVEL MULTipeptIDE REGIMEN FOR THE TREATMENT OF AUTISTIC SPECTRUM, BEHAVIORAL, EMOTIONAL AND VISCERAL INFLAMMATION/AUTOIMMUNE DISORDERS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.131

We, Martha G. Welch, David A. Ruggiero, and Muhammad Anwar, hereby declare as follows:

1. We are co-inventors of the subject matter claimed in the patent application having U. S. Serial No. 10/799,941.
2. We have been informed that the claims now pending in this application are directed to a pharmaceutical composition comprising a therapeutically effective amount of secretin in combination with a therapeutically effective amount of oxytocin.
3. We have been informed that pending claims of the '941 application have been rejected by the patent Examiner over Hollander (U.S. Pub. No. 2006/0105939) in view of an on-line NIH news alert ("The Use of Secretin to Treat Autism" Internet document), and further in view of Swain (E. Swain, Pharmaceutical and Medical Packaging News) and PIERCE (PIERCE Technical Resource Sheet TR0043.0 "Protein Stability and Storage").
4. We conceived of the pharmaceutical composition claimed in the '941 application at least prior to August 17, 2001 as evidenced by the experiments and notes included in the laboratory notebooks attached. A redacted copy of relevant lab notebook entries is attached as **Exhibit A**.
5. We diligently reduced the claimed invention to practice.
6. The claimed invention was conceived and reduced to practice in the United States.

7. The work described within this Declaration was performed by us, or on our behalf under our supervision.

8. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent in which this declaration is made.

Date: _____

Martha G. Welch

Date: 9-18-08

David A. Ruggiero
David A. Ruggiero

Date: _____

Muhammad Anwar

7. The work described within this Declaration was performed by us, or on our behalf under our supervision.

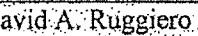
8. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent in which this declaration is made.

Date: 9/16/68



Martha G. Welch

Date: _____



David A. Ruggiero

Date: 9-16-08



Muhammad Anwar

EXHIBIT A

SECRETIN

S-1

Animal wt 250-275 g castrated 4 days before by lateral V AP+L mm 1+1.8 D-3 (Cx)

Secretin 10 μ g/5 μ l in PB 0.1M

Control 10 μ l PB 0.1M
Both injections \rightarrow 10 μ l all at once slowly
waited 3 hrs.

Perfused 4% PFA

overnight 10% sucrose

CFOS + Niels.

control animal processed (same wt) on

S-2

Animal wt 250-275 g castrated 4 days before by
as above

Secretin (5 μ g/5 μ l \times 3) at 15 min interval
PB (CONT) (5 μ l \times 3)

waited 3 hrs

Perfused 4% PFA

CFOS + Niels.

S-3

Anti Secretin Ab Test:

15,000

2 dil. dose

SECRETIN

S-4

colchicine (Rat wt 275 g)

5 μg in 10 μl saline

injected slowly

survival 24 hrs

Perfusion 4% PFA

X Secretin 1:5000

6 slides

S-5

colchicine (Rat wt >75 g)

5 μg in 10 μl saline

injected

as above

Oxy 1:5000

Secretin 1:5000

Double/ Secretin DAB oxy Gold

Control 1:5000 normal Rabbit Serum

S-6

Rat wt 455 g (6 months old)

injected 4 days before injection

Secretin 5 μg / 5 μl PB a.i. x 3 interval 15 min
waited 3 hrs before perfusion

Perfused 4% PFA

Day 1

cFos 1:8000

(to be stained for NiCf)

double

cFos / oxy 1:5000

Used Biotin/ABC for cFos

cFos / Secretin 1:5000

Used Peroxidase
conjugated IgG

cFos / SHT 1:1000

4 μg/ml

oxy, Sec, SHT

SECRETIN

S-7 (Rate 250/275g) cannulation 5 days before injections

AP = +7.0 mm

L = +1.8 mm

D = -3.0 mm (from SG)

~~Double Dose~~

Secretin 10ng/su x 3 (15 min interval)

CONTROL

(no injection)

waited 2 hrs

Perfusion 4% PFA overnight fixative

Both animal entire brains to Frontal six 3mm

T spinal cord

Adrenal

Pituitary & Pineal

processed for

cFos - Nissl

cFos - Oxy

cFos - Kcacdini limited to PVN area

cFos - CRH

DATE

TOPIC: SHR / WKY

TS - 13

Perfused

Processed

SHR

WKY

Medulla

cFos/Nissl

cFos/Nissl

cFos/PNMT

cFos/PNMT

cFos/Ri

cFos/Ri

Forebrain

cFos/Nissl

cFos/Nissl

cFos/Ri

cFos/Ri

cFos/TH

cFos/TH

cFos/Secretin

cFos/Secretin

Ti

cFos/TH

cFos/TH

CP-52 (CONT)

(Perfused)

Sectioned 16 μ Mixed \rightarrow Saline Pups - 21.72g Mixed cocaine 30.3g

Post fixed overnight with 3% PFA in 10% sucrose

(Perfused) 4% PFA overnight 16% Sucrose

Saline 39.50g cocaine 49.05g

cut coronal 30 μ

Order of collection & Processing

- ① Adrenergic
- ④ TH
- ② c-Fos
- ⑤ PNMT
- ③ GRIBA
- ⑥ Seizure
- ⑦ oxytocin

Adrenal gland c-Fos

c-Fos/ TH

c-Fos/ PNMT

Heart frozen for c-Fos/ TH

DATE:

TOPIC: Secretin/Oxy

CP-53

P-13 mother arrived on Tue
Plug

ONE DAY OLD (ex) stressed by separation 3 min
Reunited 15 min
Separated 3 min
Reunited 15 min
Separated 3 min
Reunited 2 1/2 hrs

(CONT) NO separation

Sagittal Sections Thru Pineal & Pituitary glands
also Pancreas.

CFOS/ Secretin Oxytinin
TH

4 DAYS OLD

in one pair Repeated the above procedure

in second pair one baby separated for longer (14 days)
survived

12 DAYS OLD

Repeated the above procedure

Day One procedure

14/20 days old

Both previously (4th day) stressed
and a new baby stressed
according to previous method and
stressed for See, Oxy, CFOS.

DATE: _____

TOPIC: Piglet (Secretin)

CP-59 | ~~Piglet~~ (only for Secretin) brain sections

6 days old ♂

Perfused 4% PFA (3 lit)

30 μm Sections (FB Sagittal Medull coronal)

PNMT 1:1000 (Medulla)

CART 1:1000 (Medulla, FB)

Sec 1:500 (chemicon FB)

Sec 1:1000 (Phoenix FB)

Oxy 1:1000 (chemicon FB)

DATE: _____

TOPIC: Hypoxia

CP-60 / PG-19

2 Right (in this side)

perfusion on

Medulla Prosecretaion

Pos / RH

Pos / RWM7

Pos / RH

Pos / SHRT

Tony Gica

PG-19

Free Respiration

Double Label for

Pos / Secretion

Pos / oxy. bind.

DATE: _____

TOPIC: IBD

CP-63

6 Rat Ext. approx. 225 mm long

4 for IBD
2 " CONT

TWS Dose

Day 0 Encore Dose

Oxy 242 mg / kg

Day 3

Encore Dose

Day 8

Alz A Pumped on

Animal	1.	CONT	saline
	2.	CONT	Oxy + Sec
"	3.	IBD	Oxy + Sec
"	4.	IBD	Oxy
"	5.	IBD	Sec
"	6.	Did not	

survived 5 days

Perfused 19/6 ml.

CP-63

IBD

Pre - 200 ml

TBS - 30 ml

inf. Ethanol. 500 ml

IBD ml - ml

1000 ml.

DATE:

TOPIC: IBG

CP-64

8 Rat IBD ~~am wt~~ \rightarrow 50g

4 cont
4 TNE.

one cont did

TAB

Dose
Doubled as in CP-63

OXY 0.84 / 100 μ l

See 0.84

DATE:

TOPIC: Ser/oxy Colchicine

CP - 65

Ser.

2 rats 2.5 μ l colchicine (15 mg / ml saline)
survived overnight

Rats died